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DOCUMENT SECTION

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Annual Report  
of the  
COMMISSION ON NEUROTROPIC VIRUS DISEASES  
of the  
Army Epidemiological Board  
in the Preventive Medicine Service, Office of The Surgeon General  
covering the period 1 April 1945 - 31 March 1946  
compiled in the absence of J. R. Paul, M.D. (Director)

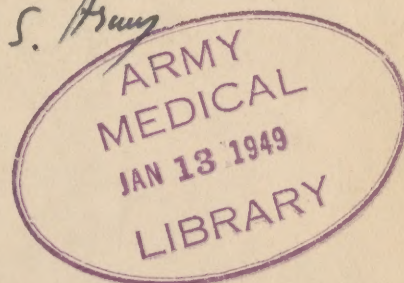
by

R. Ward, M.D.  
Acting Director 15 February - 15 March 1946

and

A. B. Sabin, M.D.  
Acting Director from 15 March 1946.

*Annual Rep. Com. Neurot. Virus Dis. U.S. Army*



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March 27th, 1946

## AREAS OF WORK AND PERSONNEL

The investigative work of the Commission has been carried out during 1945-1946 at four stations:

1) New Haven, Conn. Yale University School of Medicine.

a. Hepatitis Laboratory, under Maj. (now Dr.) W. P. Havens, Jr.

b. Poliomyelitis Laboratory, under Drs. Melnick, Wenner and Horstmann.

2) Cincinnati, Ohio. The Children's Hospital Research Foundation and University of Cincinnati College of Medicine.

The Dengue and Sandfly Fever Laboratory which has also been concerned with Japanese B encephalitis, under Lt. Col. (now Dr.) A. B. Sabin, director, and Capt. R. W. Schlesinger, M.D., and Capt. W. G. Jahnes, Sn.C.

3) San Francisco, Calif. The G. W. Hooper Foundation, University of California.

The Encephalitis Laboratory, under Dr. W. McD. Hammon, director, and Dr. W. C. Reeves and W. N. Mack.

4) New York City. Rockefeller Institute.

The Encephalitis Laboratory, under Drs. P. K. Olitsky and J. Casals.

The following members and assistants of the Commission have been separated from the Army during the past year:

1) Lt. Colonel A. B. Sabin, November 1945.

2) Major W. P. Havens, Jr., January 1946.

3) Captain W. G. Jahnes, December 1945.

4) Captain R. W. Schlesinger, April 1946

Dr. P. K. Olitsky's contract with the Commission was terminated as of 1 January 1946. Occasion is taken at this time to express great appreciation to Dr. Olitsky and Dr. Casals who, although they were not members, have contributed greatly to the work of the Commission.



2.

## ACTIVITIES IN 1945-1946

A summary of the work done from 1941 to 1946 has been presented in the form of a "History of the Neurotropic Virus Disease Commission". Before departing for Tokyo in February 1946, Dr. Paul left the following statement for this report:

"With the cessation of hostilities, the task of the Commission has been one of summarizing, completing the projects initiated under war conditions, and reconversion." Some idea of the difficulty of fulfilling this task may be gained from the following activities of the Commission since the cessation of hostilities:

- a) Intensive work in the laboratories of Doctors Hammon and Sabin on the specimens they brought back from the encephalitis outbreak on Okinawa, with special reference to the problems of serological diagnosis and epidemiology of Japanese encephalitis.
- b) Studies on unidentified viruses from Japan in Dr. Sabin's laboratory.
- c) Isolation and identification of dengue viruses from an outbreak in India, and the pursuit of new developments in the laboratory studies of dengue.
- d) An expedition to Panama in January and February 1946 to study the fevers of undetermined etiology.
- e) The departure in February 1946 of Doctors Paul and Hammon for Japan at the request of Col. Crawford F. Sams, M.C., Chief, Public Health and Welfare Section, SCAP, GHQ, AFPAC, on a special mission in connection with plans for the control of Japanese B encephalitis in Japan and Korea during the summer of 1946.

Japanese B Encephalitis.—An outbreak of encephalitis on Okinawa which began

in the native population early in July 1945 and later affected military personnel, was studied by members of the Commission (Lt. Col. Sabin, July 30 to Sept. 10, and Drs. Hammon and Reeves, Aug. 26 to Sept. 20), as well as by a team of the Naval Medical Research Unit No. 2 which was first on the scene. The etiology was established



as Japanese B virus by serological methods and by the isolation and identification of a strain of virus from a fatal case. Attempts to isolate virus from the blood and cerebrospinal fluid yielded negative results. Although only a fraction of the native cases were located, approximately 120 patients, predominantly children, were clinically diagnosed as encephalitis; 33 of these patients died. The great majority of the 38 military personnel that were considered as possible cases had very mild signs and symptoms; in only 8 of these--all with severe or moderately severe manifestations of encephalitis--was it possible to make a definite serological diagnosis of Japanese B virus infection; in the two fatal cases the diagnosis of encephalitis was made histologically and small amounts of antibody for Japanese B virus were demonstrated in serum obtained from them several days before death.

The decision to vaccinate the military personnel stationed near native foci of infection was reached by the local command early in August at a time when the future course of the outbreak could not be predicted. The mouse brain vaccine manufactured in the preceding 6 months was used. Reports are available on 53,139 individuals who received the two doses (2 cc. each) of vaccine and 2,274 individuals who had only one dose. While enough vaccine for about 77,000 individuals was distributed, the exact number vaccinated is not known because reports were not obtained from units which departed for Korea and Japan. No reactions suggesting a demyelinating encephalopathy were observed, but 5 cases of a polyneuritis-like syndrome were recorded. Nineteen instances of allergic reactions, all with good response to adrenalin, were also reported. However, owing to the disturbed conditions on Okinawa and the natural course of the epidemic no evidence was obtained either for or against the protective effect of the vaccine.

Among the observations of epidemiological significance, the following may be mentioned: a) The suspicion that Okinawa was an endemic focus was confirmed by finding neutralizing antibodies in 90 per cent of the natives (without history of encephalitis) over 20 years of age (group of 30), in 50 per cent in the age group of 10 to 19, and in none of 16 individuals in the age group of 1 to 9; b) neutralizing



antibodies were also found in the horse, goat, and cow but not in chickens. The failure to isolate virus from Culex quinquefasciatus, the predominant mosquito during the outbreak, agrees well with the absence of antibodies in chickens, and suggests that some other mosquito, biting the larger domestic animals and human beings by preference, might have been the vector on Okinawa.

Dengue.--Two additional strains of dengue virus were isolated during the past year from serum specimens brought from India by Col. Herman L. Blumgart. Both of these strains proved to be immunologically identical with the original strain of virus isolated from Hawaii. Repeated attempts were made to isolate a virus by inoculation of human subjects with sera obtained in the Philippines and Okinawa during outbreaks of febrile illnesses diagnosed as atypical dengue, but without success.

The Hawaii strain of dengue virus has now undergone 32 consecutive passages in mice, with an increase in its intracerebral LD<sub>50</sub> titer from  $10^{-2}$  to  $10^{-4}$ . Even after this thorough adaptation, the virus is pathogenic for mice (by the intracerebral and not the intraperitoneal route), but not at all for cotton rats, hamsters, guinea pigs or rabbits--a host range by which it can be distinguished from other known viruses. The primary attack in mice is on the neurones, while biopsies of the human skin lesions suggest that in man the primary attack is on the blood vessels. The identity of the mouse passaged virus has now been proved by many human and serological tests. In the process of this adaptation to mice the virus underwent a change or mutation, whereby it has lost the capacity to produce the severe illness and protracted fever characteristic of the original disease, but has retained the capacity to produce the rash, and subsequent immunity to the unmodified dengue virus. The extract from a single mouse brain has been found to contain at least 10,000 human immunizing doses, and an effective lyophilized vaccine consisting of this modified mouse virus has been prepared.

After 16 to 18 passages in mice, but not with earlier passages, it proved possible to propagate the virus in embryonated <sup>y</sup>eggs when 5-day old embryos were used for inoculation and a period of 8 to 10 days at 35° C. for incubation. A satisfactory



mouse neutralization test has now been worked out and its use has already yielded data of epidemiologic significance. The most interesting advance in this work was made during the last few months when it was found that the 3 New Guinea strains of dengue virus which are immunologically different from all the others, and which for 2 years resisted adaptation to mice, could finally be propagated when the initial passages were made in "dba" mice, a special, genetically homogeneous breed.

Two strains of mouse virus obtained from Dr. Ishii in Japan, who stated that they represented Japanese dengue viruses, received a systematic investigation. They were found to be two strains of the same virus, but entirely different from the dengue viruses isolated by us from various parts of the Pacific and India, as regards host range, particle size, and immunological identity; furthermore, the properties of this Japanese virus did not correspond to any other known virus.

Infectious Hepatitis and Homologous Serum Jaundice (Dr. Havens).--During the past year at the Hepatitis Laboratory at Yale, 60 human volunteers (conscientious objectors and prisoners\*) have been inoculated during experiments in the transmission of infectious hepatitis and homologous serum jaundice.

Comparative studies of the behaviour of our two strains of virus in man have demonstrated certain similarities and differences which are recorded in the following table:

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\*This work has been made possible by the collaboration of the following agencies: Yale University School of Medicine; the Bureau of Personnel, State of Connecticut; the Middletown and Norwich State Hospitals of Connecticut; the Federal Correctional Institution, Danbury, Ct.; the U. S. Public Health Service; the National Association for Religious Objectors; and the Connecticut State Prison, Wethersfield, Ct.



Icterogenic Agent	INFECTIOUS HEPATITIS	HOMOLOGOUS SERUM JAUNDICE
1. Filtrability.	Seitz EK.	Seitz EK.
2. Resistance to heat.	56°C. for 30 minutes.	56°C. 60 minutes.
3. Host range.	Man.	Man.
4. Incubation Period. (Days)	15 - 34	56 - 134
5. Presence in Stool.	Acute phase.	Not demonstrated.
6. Presence in Serum.	Acute phase.	Incubation period and acute phase.
7. Route of infection. (Experimental)	Parenteral or oral inocu- lation	Parenteral inoculation.
8. Immunity.		
a. Homologous	Present.	Not tested.
b. Heterologous	Not tested.	None.

Clinical studies of experimental infectious hepatitis have also been made. A regular pattern of hematologic response with leukopenia, lymphopenia, and neutropenia is associated with the appearance of fever. Later in the pre-icteric period a relative lymphocytosis with numerous atypical cells is a common phenomenon. Gastroscopic examinations were made on 6 patients and revealed an acute superficial gastritis during the acute phase of disease with subsidence in convalescence. Attempts to correlate these changes with roentgenographic changes were unsuccessful and the occasional roentgenographic evidence of gastritis or duodenitis was equivocal.

An attempt to transmit our strain of infectious hepatitis to 4 young (2-3 years) chimpanzees was unsuccessful.

An effort was made to test two lots of gamma globulin during an outbreak of infectious hepatitis in a school for mental defectives in Southbury, Connecticut, to determine: a) the minimum effective prophylactic dose of one lot, and b) the prophylactic effect of the second lot. The epidemic was too brief for a satisfactory test.

Poliomyelitis. A. Isolation of Virus from Army Material.---During 1945-46 material was received mostly from the Philippines consisting of 6 separate lots of



stool specimens and specimens of spinal cord and brain in glycerin. Stool specimens were also received from Italy. From these materials poliomyelitis virus was isolated 11 times: 5 times from stool specimens sent from the Philippines, and 5 times from human central nervous system and once from monkey (passage) brain from the Philippines. Information derived from these tests, because of the long time required for their completion, has had little clinical application, although it has proved of epidemiological value.

B. The outbreak at Ft. McClellan.--During March and April 1945 an unusual, sharp, and moderately serious outbreak of poliomyelitis occurred at Ft. McClellan, Alabama. The seriousness was due to the fact that of the 17 cases, 10 were bulbar in type, and 3 of these were fatal.\* The chronological grouping of the cases suggested that a large amount of virus might have been rather suddenly introduced into the camp population. The cases were mostly scattered throughout the camp, not 1 patient knowing another patient. Tests on water, milk, ice cream, sludge and flies collected towards the end of the outbreak were negative for poliomyelitis virus. No definite answer could be reached as to the mode of spread.

C. Fly abatement project (Drs. Melnick and Ward).--Although poliomyelitis virus has been detected in flies and in fly-contaminated food collected in epidemic areas, these findings do not establish the fly as a vector of poliomyelitis. It was thought that, if a prompt and substantial reduction in the fly population could be achieved in part of a poliomyelitis epidemic area, information might be forthcoming on the role played by the fly in spreading this disease. D.D.T. was applied accordingly in two epidemic areas, each being about 4 square miles and inhabited by some 67,000 and 27,000 people, respectively; a temporary (5 to 20 days) reduction in flies was achieved in both areas. No conclusions can be drawn, however, regarding the effect of this spraying on the epidemic because in one area (Paterson, N. J.) the epidemic failed to progress following the spraying of D.D.T. in either the

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\*Poliomyelitis virus was isolated from the c.n.s. of one of the fatal cases.



sprayed or the control areas, and in the second area (Rockford, Ill.), D.D.T. was applied in the fifth week, at a time when the epidemic was subsiding.

### Encephalitis and Poliomyelitis in the West (Dr. Hammon).

#### 1. Epidemiological investigations in Kern County, California.

An etiologic diagnosis could be made in only 10% of 57 cases of supposed neurotropic virus infections in Kern County General Hospital during 1944. Four cases of St. Louis encephalitis (SLE) and 2 of western equine encephalitis (WEE) were diagnosed by demonstrating an increase in serum neutralizing antibody titre. Poliomyelitis virus was not detected in a number of other patients.

The year 1945 was one of epidemic proportions for encephalitis in horse and man. WEE virus has been isolated from 1 fatal case and 17 cases have been diagnosed as WEE chiefly by the complement fixation test. The latter also was the means of diagnosing two cases of mumps meningo-encephalitis without parotitis.

#### 2. D.D.T. Encephalitis Control Program.

1) The use of residual D.D.T. in buildings which serve as resting places for mosquitoes was quite effective, causing at least a 90 per cent reduction of the total calculated expected Culex populations in those buildings.

2) D.D.T. in diesel oil (200 mg./sq.ft.) on unpainted dry wood is carried deep below the surface leaving almost no detectable residual after a very short period of time (less than two months). A xylene-water emulsion produced a better result on these surfaces.

3) Any area left unsprayed (i.e., upper surface of rafters, between shingles, inside of box) or the unsprayed surface of any article introduced after spraying could usually be found harboring mosquitoes in fairly large numbers.

4) Intensive larval control measures plus residual D.D.T. spraying of most buildings has only a moderate effect on reducing the population of Culex mosquitoes over the area as a whole.

5) WEE virus infection rates remained high in Culex tarsalis within the treated areas.



6) A serum neutralization test survey of young chickens failed to demonstrate that a reduction in the number of resting mosquitoes in chicken houses resulted in a proportionate decrease in the incidence of infection in these birds.

7) A similar survey of domestic and wild birds, wild rabbits, and ground squirrels indicated widespread infection in these groups. The pigeon and meadowlark had very high rates.

8) Although one case of WEE in a horse occurred in the center of the sprayed area, horse and human clinical infection rates for the county were too low to lend significance to the absence of other cases in the areas studied.

3. The "California" virus. This new agent, isolated from mosquitoes in 1944, was shown to be unrelated to Theiler's virus (mouse encephalomyelitis). Evidence has been acquired both from the field and laboratory suggesting that ground squirrels and rabbits may serve as its reservoirs.

4. Experiments with Japanese B encephalitis virus (Jap. B). After 50 egg passages of Jap. B virus, the whole embryo yielded a titre of  $10^{-8.5}$  in mice. A satisfactory complement fixing antigen was made from such whole embryos.

Chickens were found (experimentally) capable of acting as temporary reservoirs. Thus, following subcutaneous injection of small amounts of virus, isolation of virus from blood was made irregularly from 48 to 168 hours. None was detected in the spleen.

5. A poliomyelitis epidemic was studied in a trailer camp at Mill Valley, California. Poliomyelitis virus was recovered from throat swabs taken on three patients early in the disease. An unusual feature of this epidemic was the age distribution. Over half the cases occurred in adults. The mode of spread in this outbreak was not elucidated.



SCIENTIFIC REPORTS  
(Unpublished)

1 April 1945 - 31 March 1946

- April Minutes - Meeting of 3 April 1945 in New York City.
- April by W. McD. Hammon. Poliomyelitis Epidemics Investigated on the West Coast. 1. U.S. Navy Receiving Barracks, Portland, Oregon. 2. Occidental College, Los Angeles, California.
- May by R. Ward, and J. R. Paul. Report on the Outbreak of Poliomyelitis at Fort McClellan, Alabama (1 March - 15 April 1945)
- June by A. B. Sabin. Vaccination of Human Beings with Modified Dengue Virus.
- July by A. B. Sabin. Human Transmission Tests with Sera from F.U.O. Cases on Leyte (Philippines) during February and March 1945.
- Aug. by R. W. Schlesinger. A. Preparation of Dengue Vaccine with Virus Propagated in Mice. B. Tests on Human Beings.
- Aug. by G. D. Gammon, and E. B. Schoenbach. Investigation of Diphtheria and Polyneuritis in MTOUSA. A Preliminary Report
- Sept. The Etiology of Infectious Hepatitis.
- Sept. by R. W. Schlesinger. Appendix I. Results of Immunity Tests to original report, "A. Preparation of Dengue Vaccine with Virus Propagated in Mice. B. Tests on Human Beings".
- Sept. by A. B. Sabin. Encephalitis in Okinawa - Report on Outbreak of the Disease among Civilian and Military Personnel During July, August, and September 1945.
- Oct. The Virus of Poliomyelitis.
- Oct. Minutes - Meeting of 18 October in New York City.
- Oct. by W. McD. Hammon. Preliminary Report on Encephalitis on Okinawa, 1945.
- Dec. by J. L. Melnick, R. Ward, D. R. Lindsay, and F. E. Lyman. Preliminary Report of Fly Abatement Studies in Urban Poliomyelitis Epidemics, 1945.
- Dec. History of the Commission on Neurotropic Virus Diseases, 1941-1945.
- Dec. by A. B. Sabin. Isolation and Identification of Two Strains of Dengue Virus from "Fever" among U.S. Army Personnel in India.
- Dec. by A. B. Sabin. Human Transmission Tests with Sera from "F.U.O." cases on Mindoro, Philippine Islands (165th Station Hospital-Lt. Col. C. B. Philip, Sn. C.), during March and April, 1945.



- Dec. by A. B. Sabin. Dengue-Like Fever on Okinawa - Human Transmission Tests.
- Jan. by A. B. Sabin. Immunological Identity of "Kalinina" and "Matsunaga" strains of Japanese B Encephalitis, obtained from Dr. Y. Kawakita and brought from Japan by Colonel Bruce Webster, 23 October 1945.
- Jan. by A. B. Sabin. Positive Serological Tests for Japanese B Encephalitis Virus with Sera from Cases of Encephalitis in Military Personnel in Philippines (July - August 1945).
- Jan. by A. B. Sabin. Properties and Identity of an Alleged Mouse-Adapted Dengue Virus Obtained from Dr. N. Ishii in Japan, 18 October, 1945.
- Feb. by W. McD. Hammon. Experience with the Complement Fixation Test in the Diagnosis of Japanese B Encephalitis Virus Infections.
- March by A. B. Sabin. Investigation of Fevers of Undetermined Origin in the Canal Zone and Republic of Panama with Special Reference to Possible Endemicity of Dengue and Phlebotomus (Sandfly) Fever.

## PUBLICATIONS

31 March 1945 - 31 March 1946

- March Havens, W.P., Jr.: Properties of the Etiologic Agent of Infectious Hepatitis. Proc. Soc. Exper. Biol. & Med., 1945, 58, 203-204.
- May Olitsky, P.K., Morgan, I.M., Schlesinger, R.W.: Vaccination with Various Western Equine Encephalomyelitis Viruses; Comparison as Antigens and as Test Inocula. Proc. Soc. Exper. Biol. & Med., 1945, 59, 93-97.
- May Ward, R., Melnick, J.L., Horstmann, D.M.: Poliomyelitis Virus in Fly-Contaminated Food Collected at an Epidemic. Science 101:491, 1945.
- June Havens, W.P., Jr.: Experiment in Cross Immunity Between Infectious Hepatitis and Homologous Serum Jaundice. Proc. Soc. Exper. Biol. & Med., 1945, 59, 148-150.
- June Morgan, Isabel M.: Quantitative Study of the Neutralization of Western Equine Encephalomyelitis Virus by its Anti-Serum and the Effect of Complement. J. Immunol., 1945, 50, 359-371.
- June Sabin, A. B., Schlesinger, R.W.: Production of Immunity to Dengue with Virus Modified by Propagation in Mice. Science, 1945, 101, 640-642.
- July Casals, J.: The Technique and Practical Applications of the Complement Fixation Test for Diagnosis of Infection with Encephalitis



Viruses. J. Bact., 1945, 50, 1-5.

- July Harmon, W. McD., Reeves, W.C., Galindo, P.: Epizootology of Western Equine Type Encephalomyelitis: Eastern Nebraska Field Survey of 1943 with Isolation of the Virus from Mosquitoes. Amer. J. Vet. Resch., 1945, 6, 145-148.
- July Paul, J.R., Havens, W.P., Jr., Sabin, A.B., Philip, C.B.: Transmission Experiments in Serum Jaundice and Infectious Hepatitis. J.A.M.A., 1945, 128, 911-915.
- Sept. Havens, W.P., Jr., Paul, J.R.: Prevention of Infectious Hepatitis with Gamma Globulin. J.A.M.A., 1945, 129, 270-272.
- Oct. Hammon, W. McD., Reeves, W.C.: Advances in the Epidemiology of the Arthropod-borne Virus Encephalitides, Including Certain Exotic Types. Amer. J. Pub. Health, 1945, 35, 994-1004.
- Oct. Hammon, W. McD., Reeves, W.C.: Certain Bacteriostatic Agents Added to Sera Used in Diagnostic Tests for Neurotropic Virus Infections. Proc. Soc. Exper. Biol. & Med., 1945, 60, 84-88.
- Oct. Havens, W.P., Jr., Ward, R.: Failure to Transmit Infectious Hepatitis to Chimpanzees. Proc. Soc. Exper. Biol. & Med., 1945, 60, 102-104.
- Oct. Olitsky, P.K., Casals, J.: Certain Affections of the Liver that Arise Spontaneously in so-called Normal Stock Albino Mice. Proc. Soc. Exper. Biol. & Med., 1945, 60, 48-51.
- Nov. Neurotropic Virus Disease Commission: The Etiology of Infectious Hepatitis. Bull. U.S. Army Med. Dept., 1945, 4, 498-499.
- Nov. Sabin, A. B.: Neurotropic Virus Diseases in the West Pacific and Far East. J. Military Med. in the Pacific, 1945, 1, 56-57.
- Nov. Sabin, A. B.: Outbreak of Encephalitis on Okinawa in 1945. Preliminary Report on Status as of 27 August 1945. Ibid, 1945, 1, 79-84.
- Nov. Hammon, W. McD., Reeves, W. C., Galindo, P.: Epidemiologic Studies of Encephalitis in the San Joaquin Valley of California, 1943, with the Isolation of Viruses from Mosquitoes. Amer. J. Hyg., 1945, 42, 299-306.
- Dec. Neurotropic Virus Disease Commission: The Virus of Poliomyelitis. Bull. U.S. Army Med. Dept., 1945, 4, 630-631.
- Dec. Casals, J., Olitsky, P.K.: Enduring Immunity following Vaccination of Mice with Formalin - Inactivated Virus of Russian Spring-Summer (Far Eastern, Tick-borne) Encephalitis. J. Exper. Med., 1945, 82, 431-443.



- Dec. Casals, J.: Heated, Avirulent Antigens for Complement-Fixation Tests with Certain Encephalitis Viruses. Science, 1945, 102, 618-619.
- Jan. Havens, W.P., Jr.: Epidemiological Studies on Infectious Hepatitis. Amer. J. Pub. Health, 1946, 36, 37-44.
- Jan. Havens, W. P., Jr., Wenner, H.A.: Infectious Hepatitis Complicated by Secondary Invasion with Salmonella. J.Clin. Invest., 1946, 25, 45-52.
- March Hammon, W. McD., Reeves, W.C.: Western Equine Encephalomyelitis Virus in the Blood of Experimentally Inoculated Chickens. J. Exper. Med., 1946, 83, 163-173.
- March Hammon, W. McD., Reeves, W.C., Izumi, E.M.: St. Louis Encephalitis in the Blood of Experimentally Inoculated Fowls and Mammals. J. Exper. Med., 1946, 83, 175-183.
- March Reeves, W.C., Hammon, W. McD.: Laboratory Transmission of Japanese B Encephalitis Virus by Seven Species (three genera) of North American Mosquitoes. J. Exper. Med., 1946, 83, 185-194.
- March Havens, W.P., Jr.: Period of Infectivity of Patients with Experimentally Induced Infectious Hepatitis. J. Exper. Med., 1946, 83, 251-258.